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Dendrimers

Antony D'Emanuele
David Attwood
Ragheb Abu-Rmaileh
University of Manchester, Manchester, U.K.

INTRODUCTION

Dendrimers are highly branched macromolecules that can be subdivided into three architectural components: a central core branched cell, interior branch cells, and branch cells possessing surface groups. The term dendrimers was coined in the early 1980s by Tomalia et al.[1] from the Greek words dendron (tree) and meros (part) and relates to the symmetrical branch-like structure of these polymers. Dendrimers are synthesized through a stepwise repetitive reaction sequence, which gives rise to different generations of the same molecule and determines the size and surface functionality of the macromolecule. The dendrimer microenvironment possesses some interesting properties. Cavities in the core structure and folding of the branches create cages and channels, which, depending upon how the dendrimer is constructed, may be either hydrophilic or hydrophobic in nature. Specific binding sites may also be incorporated. The surface groups of dendrimers are amenable to modification and can be tailored for specific applications. The dendrimer architecture, therefore, permits control over properties such as shape, size, density, polarity, reactivity, and solubility. The structure of a generation 3 polyamidoamine (PAMAM) dendrimer is shown in Fig. 1. Dendrimers can be very large; PAMAM dendrimers have been synthesized with a diameter in excess of 10 nm. Fig. 2 compares the size of a series of PAMAM dendrimers to a number of biological structures. Table 1 gives the relationship between generation and dendrimer size, molecular weight and number of surface groups.[2] As can be seen, an increase in dendrimer generation results in a doubling in the number of surface groups.

Although the synthesis of dendrimer-like structures was initially described in 1978,[3] it is only since the 1990s that there has been an intense interest in these polymers, partly attributable to the availability of a commercial source of PAMAM dendrimers.

SYNTHESIS

Dendrimers may be prepared by either a convergent[4–6] or a divergent approach.[1] Much of the work on dendrimers has been based on the commercially available Starburst® PAMAM dendrimers,[1,7] which may be synthesized from an ammonia or ethylenediamine core (EDA), and possess an amidoamine branching structure. This family of dendrimers was introduced by Tomalia et al. in 1985[1] and are prepared by the divergent approach, where the branching dendritic structure is built up from a central core. PAMAM dendrimers are synthesized by an iterative process involving two reactions: Michael addition followed by amidation. The first iteration of these two reactions results in the formation of a zero-generation (G0) dendrimer, the subsequent addition–amidation cycles each result in growth and the formation of a higher generation (Fig. 3), this synthetic cycle yields a full-generation (amine terminated) dendrimer. Cessation of the reaction after the Michael addition results in the eventual formation of a half-generation (carboxyl terminated) dendrimer. The growth of a dendrimer is self-limiting, and governed by steric hindrance arising from the introduction of numerous surface groups.[8,9] The convergent method of synthesis involves the initial creation of the dendrimer branches, followed by assembly to form the dendrimer. The convergent method produces a system with a low polydispersity, while the divergent method can produce dendritic structures with defects arising from incomplete reactions.[10] However, the divergent method has the advantage of being able to yield higher generation dendrimers. The methods of synthesis and their relative merits have been reviewed by Dykes.[11]

Three structural components are common to all dendrimers: a core unit, peripheral groups, and the multiple branching units that span the two. The core unit in dendrimers is usually an important part of the structure as it covalently links the dendritic "wedges" (dendrons). However, cored dendrimers have been synthesized in which surface crosslinking maintains the integrity of the dendritic structure.[12]

The preparation of structurally perfect dendrimers traditionally can be time-consuming due to the required repetitive coupling and activation steps, and the necessity for extensive purification.[13] PAMAM dendrimers are just one of several types of dendrimers with pharmaceutical applications that have been synthesized. Poly(aryl ether) dendrimers were developed by Hawker and Fréchet and were the basis of unimolecular micelles and cancer drug conjugates.[4,14,15] Wang, Zeng, and Zimmerman[16]

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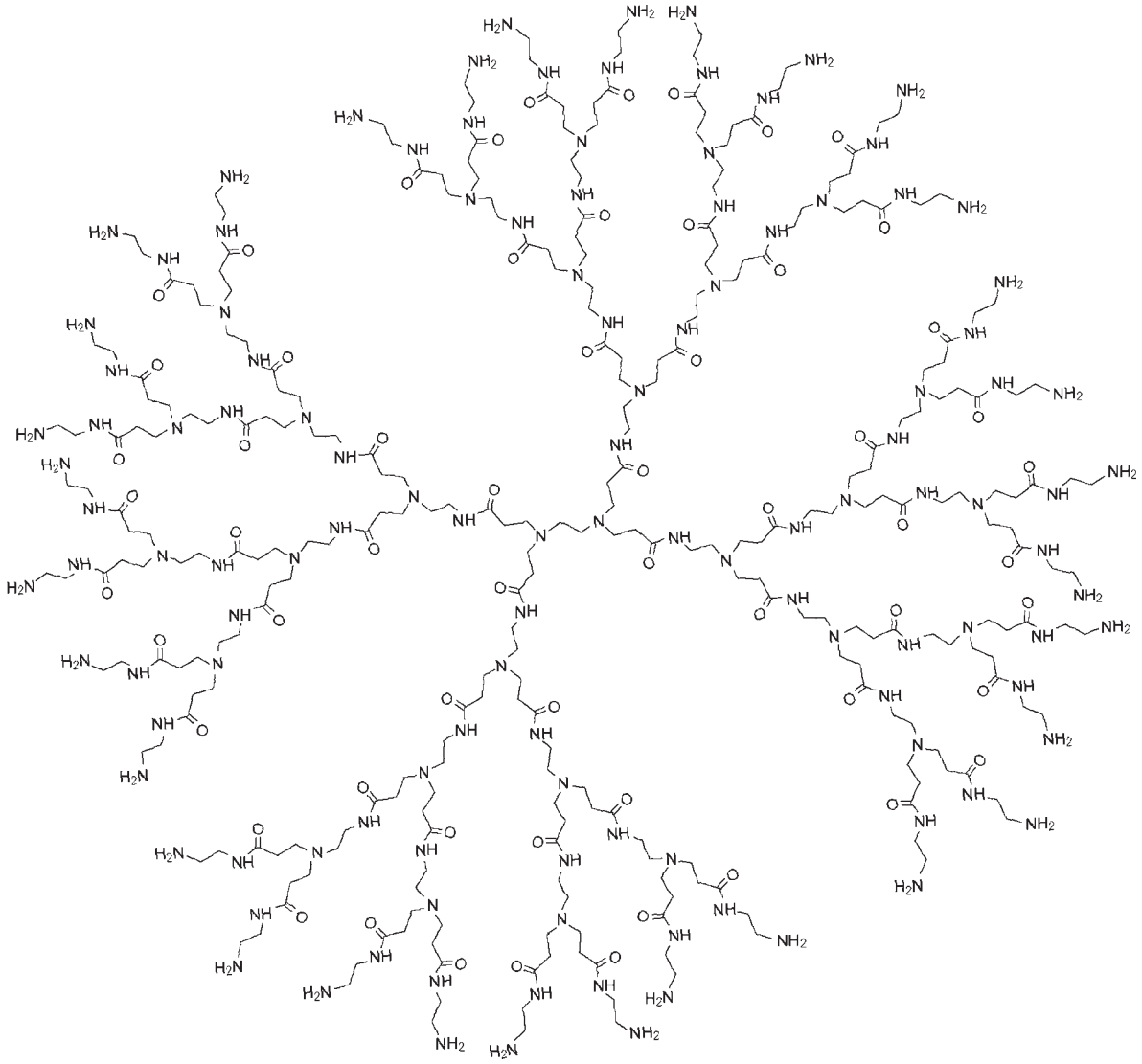

Fig. 1 Structure of a G3 PAMAM dendrimer. (From Ref. [110], © 2000 Elsevier Science.)

reported the synthesis of poly(phenylacetylene) and poly(benzyl ether) (PBE) dendrimers to encapsulate benzamidinium guests by hydrogen bonding. Jansen, de Brabander-van den Berg, and Meijer[17] described the synthesis of poly(propyleneimine)-based dendrimers and their applications in the encapsulation of guest molecules. Numerous other dendrimer families have been synthesized with a wide range of applications.[18–20]

Several peptide dendrimers have been reported,[21–25] a major application of which is for the preparation of multiple antigen peptides (MAPs). For example, Tam[26] used the poly(lysine) platform to prepare a MAP. These peptides are used to activate the immune system to produce large numbers of antipeptide antibodies, and their use avoids the immunogenicity and the other disadvantages associated with conventional antigenic systems.

Dendrimer structure is often confirmed with a variety of techniques, including ^{1}H- and ^{13}C-NMR, mass spectrometry, size-exclusion chromatography, high performance liquid chromatography, electrophoresis, elemental analysis, and thermal analysis.[14,27–35]

PHYSICOCHEMICAL PROPERTIES

Unlike classical polymers, dendrimers have a high degree of molecular uniformity, narrow molecular weight distribution, specific size and shape characteristics, and

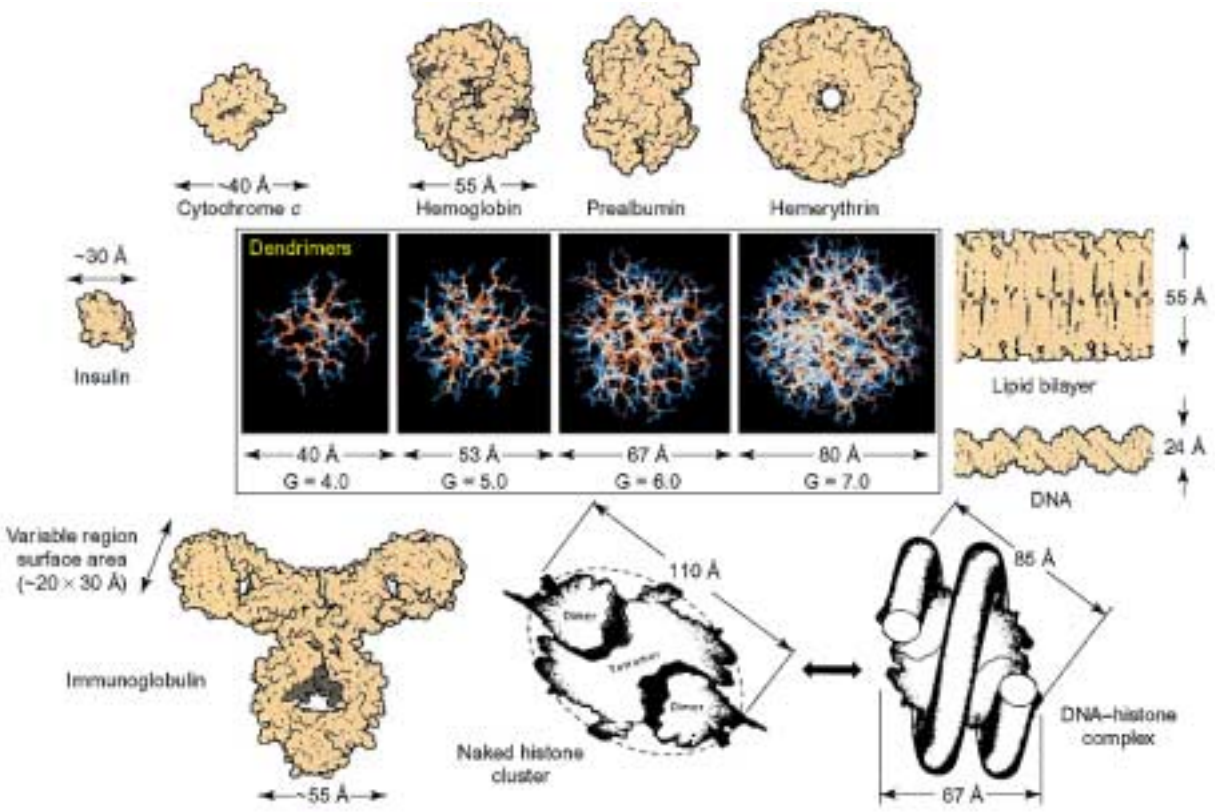

Fig. 2 A dimensionally scaled comparison of a series of PAMAM dendrimers (NH_3 core) with a variety of proteins and bioassemblies. (From Ref. [7], © 2001 Elsevier Science.)

a highly functional terminal surface. The branching nature of the structure can lead to large 3-D globular structures, which at high molecular weights may approximate spheres. These structures are relatively fixed, in marked contrast to linear polymers that are random coils and, depending on the solvent, may adopt a variety of configurations.

Many of the physical properties of dendrimers may be predicted, at least qualitatively, from molecular modeling of the growth process. With the PAMAM dendrimers,

Table 1 Physical characteristics of PAMAM dendrimers (EDA core)

Generation	Molecular weight	Measured diameter (nm)	Surface groups
0	517	1.5	4
1	1,430	2.2	8
2	3,256	2.9	16
3	6,909	3.6	32
4	14,215	4.5	64
5	28,826	5.4	128
6	58,048	6.7	256
7	116,493	8.1	512
8	233,383	9.7	1024
9	467,162	11.4	2048
10	934,720	13.5	4096

(From Ref. [2].)

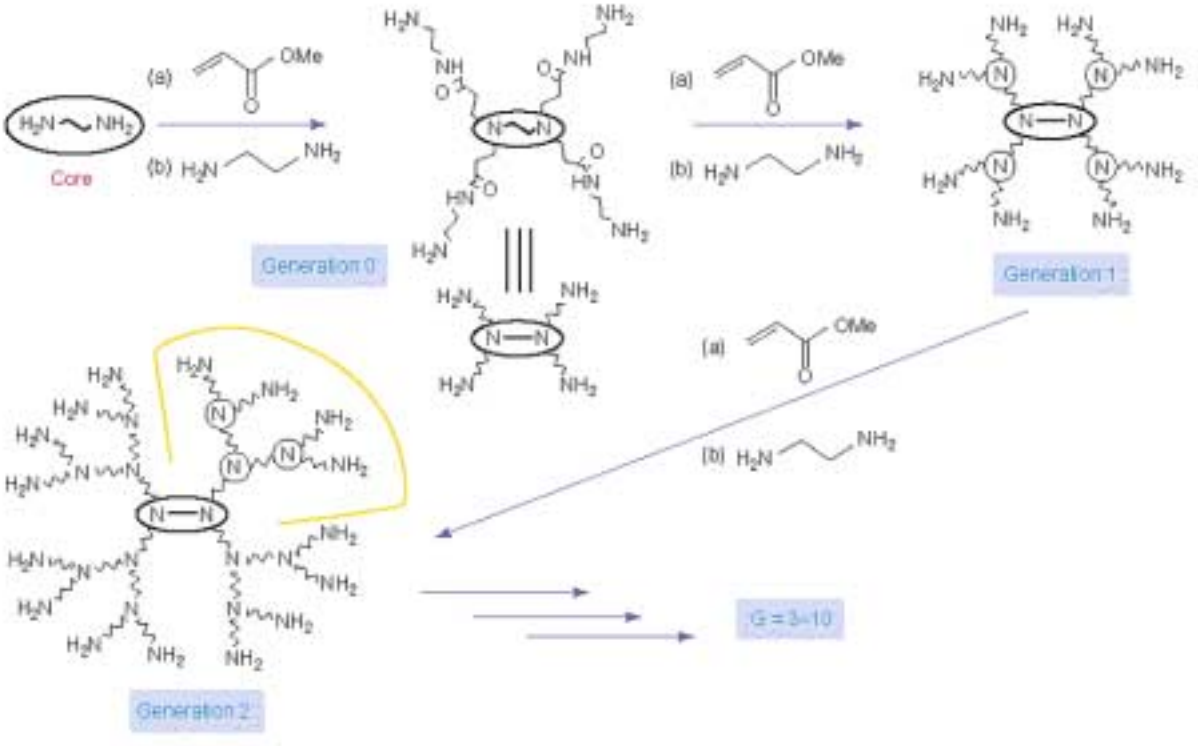

Fig. 3 Synthesis of a PAMAM dendrimer: exhaustive Michael addition of amino groups with methyl acrylate, followed by amidation of the resulting esters with EDA. (From Ref. [7], © 2001 Elsevier Science.)

a fully developed dendrimer structure first appears only after dendrimer growth to at least G1.5, since it is not until the point that the dendrimer contains all three branch cell components, i.e., the core, interior, and surface branch cells. This transition from lightly branched structures to fully developed dendriditic structure is referred to as the critical branching stage.[36] Because of the progressive growth pattern, the dendrimers are constructed in a precise manner and a linear increase of the radii of the fully developed dendrimers with increase of generation is expected. However, because the surface cells amplify according to a geometric progression with increasing generation, it is clear that ideal growth cannot continue indefinitely and there will be a critical generation at which the reacting dendrimer surface will not have sufficient space to accommodate all of the required new units. This stage is referred to as the de Gennes dense-packed state and occurs around G7 in the PAMAM dendrimers.[37] Dendritic growth beyond this point is of course possible but leads to products of imperfect structure because not all of the surface groups are able to participate in reaction due to steric effects. Such defective generations have been called "hairy dendrimers."

Similar molecular modeling of the 3-D character of the polyether dendrimers, [37] on the other hand, shows that steric crowding of the surface branches becomes prohibitively high on approaching G4. As a consequence, it is predicted that the polyether dendrimers will be denser with far fewer internal cavities than the PAMAM series. The PAMAM dendrimers have much higher internal surface area and solvent-filled volume than the polyether dendrimers. Within the PAMAM dendrimer spheres formed at $G > 4$, the solvent accessible surface increases with generations so that the fraction of the internal molecular surface increases from about 29% of the total solvent accessible surface at G4, to 69% for G5, and about 124% for G6.[37] Thus, at G6 there is more internal surface area than external surface area. For polyether dendrimers on the other hand, the internal surface area reaches a maximum of approximately 20% for G3 and G4. The possibility of binding or entrapping small molecules in these cavities mimics the drug delivery attributes that are offered by liposomes.

Dendrimer Size and Shape

Computer-simulated modeling of the structures of the PAMAM dendrimers[37–39] highlights changes in external appearance with increase in generation (Fig. 2), through a continuum of molecular shapes ranging from open

amorphous shape for the early generations (G0 to G3) to a more tangled spheroidal network for the fully developed structures at $G > 4$. These shape changes are a consequence of the tethered steric constraints imposed on the developing branches. In Fig. 4, these shape changes are expressed in terms of the aspect ratios of the corresponding longest and shortest principle moments. Experimental determination of the exact shape of dendrimers presents difficulties mainly because most of the commonly used techniques are at their limits of reliability in the size range involved.

Very few experimentally determined transmission electron microscope (TEM) images of dendrimers have been published in the literature, probably because their size range and their fragile organic composition make the resolution of such objects by electron beam techniques very difficult.[40] Quality images of dendrimer molecules may provide insight into the actual uniformity, sphericity, and hollowness of the macromolecules. TEM studies on PAMAM dendrimers by Tomalia et al.[1] showed highly monodisperse spheroids. For example, images of the sodium salt of G3.5 dendrimer showed that almost 90% of all particles had diameters ranging within 10% of the average value determined by computer simulations. Jackson et al.[41,42] examined positively stained PAMAM dendrimers of G5 to G10 using this technique. The shapes of the stained molecules were shown to be spherical for G7 to G10 with some molecules showing "edges." For G5 and G6, the resolution of these molecules was less than with larger dendrimers because smaller molecules took up less amount of stain. The mean diameters of the dendrimers measured by TEM compared well with measurements made with small-angle x-ray scattering (SAXS).

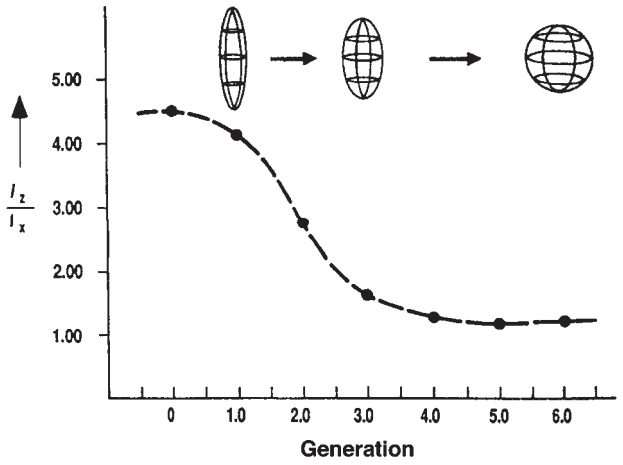

Fig. 4 Comparison of the change in PAMAM dendrimer morphology (aspect ratio I_z/I_x) as a function of generation. (From Ref. [37], © 1990 Wiley-VCH.)

Dendritic structures have been studied on a variety of surfaces including glass,[43] graphite,[43,44] and charged solid surfaces[45,46] using atomic force microscopy (AFM). In a recent study, Zhang et al.[47] reported the topographical imaging of well-separated, individual polyphenylene dendrimer molecules and their aggregates on mica surfaces using noncontact AFM techniques. The observed height was in good agreement with the size of a single dendrimer molecule as calculated by molecular dynamics simulation. In addition, pulse force mode AFM was used to study the stiffness and adhesion properties of the individual dendrimer molecules to this surface and these were related to the molecular structure and the chemical nature of the outer surface of the dendrimers.

Experimental measurements of the hydrodynamic radius of PAMAM dendrimers with ammonia or EDA cores by size exclusion chromatography and viscometry[37,39] have shown the expected linear increase of hydrodynamic radius with generation beyond G3. The values were intermediate between the calculated theoretical maximum values that would result from maximum extension of all branches, and the minimum value allowed by acceptable packing of the atomic mass units around the initiator core. The expanded dendrimer would be expected to occur with more ideal solvents; the compressed form would be more likely in theta solvents. A small-angle neutron scattering (SANS) study of the effect of solvent quality on the molecular dimensions of G5 and G8 PAMAM dendrimers[48,49] showed a decrease of the radius of gyration of the dendrimers by approximately 10% with decreasing solvent quality. The same studies showed that the dimensions of these two dendrimers were constant to within 5% over the temperature range -10 to 50°C.

PAMAM dendrimers have been obtained as very monodisperse samples by careful removal by ultrafiltration of propagating agents and any bridged forms. Measurement by a variety of techniques, notably size exclusion chromatography and low-angle laser light scattering,[37] SANS and SAXS,[50,51] and intrinsic viscosity measurement[39] have shown polydispersity indices for PAMAM dendrimers in the range 1.01–1.08, indicative of a relatively monodisperse distribution of dendrimer sizes in solution. A similar monodispersity of size was noted from SAXS measurements[52] on dilute methanolic solutions of G3 to G10 PAMAM dendrimers and poly(propyleneimine) dendrimers made with primary amines built around a diaminobutane core (DAB-*dendr*-$(NH_2)_x$, $x = 16$, 32, and 64). In contrast, dilute solutions of polyol hyperbranched polymers exhibited scattering indicative of the inherent irregularity of internal segment densities and of overall size, giving more polydisperse solutions. SANS measurements on poly(propyleneimine) dendrimers terminated by primary

amines, DAB-*dendr*-$(PA)_{32}$ and DAB-*dendr*-$(PA)_{64}$, in methanol over a wide concentration range (dendrimer mass fraction between 0.01 and 0.80) have been reported by Topp et al.[53] At concentrations at which the swollen dendrimer volume fraction was below 0.64 (the critical volume fraction for close packing of hard spheres), which corresponds to a dendrimer weight fraction of 0.25, the dendrimers in solution behaved as a dispersion of uniform soft spheres with no significant interpenetration between the segments of different dendrimers. At higher concentrations, however, the dendrimers "collapsed," their size decreasing with increase of number density so that the volume fraction of the solution was maintained at approximately 0.64.

Surface Properties of Dendrimers

The surface properties of dendrimers are specific to the functional terminal groups that make up the surface of the dendrimers, which can be either reactive or passive moieties or even a combination of both. The type and number of functional groups and their ionization characteristics may affect dendrimer solution properties in various ways, e.g., by changing the solubility, potential for aggregation, and inter-dendrimer charge interaction. A dendrimer surface may contain multiple copies of a particular functional group and so would be an ideal molecule for substrate binding. Under conditions where the functional groups are ionized, the dendrimer becomes a macromolecular polyelectrolyte and as such it will interact strongly with oppositely charged particles. Polyelectrolyte dendrimers have been shown to adsorb strongly at various interfaces such as alumina/water and silica/water as well as associating with proteins or DNA.[54–57]

Although dendrimers possessing a wide variety of functional groups have been synthesized, the most studied are those with amine or carboxyl groups. A titration curve of a 2% aqueous solution of a G4 PAMAM dendrimer against 0.2 *M* HCl[58] shows two distinct inflection points that are indicative of the titratable terminal primary amines (pK_a 10.7) and the interior amines (pK_a 6.5). The titration characteristics have been confirmed by observation of ^{13}C chemical shifts.[1] The surface potential of the G4 PAMAM dendrimer calculated from titration curves is 63 mV.[58]

Charge Interactions and Aggregation

A pronounced influence of pH and added electrolyte on the charge interactions between dendrimers with ionizable groups is expected in aqueous solution. Briber et al.[50] investigated the intermolecular interactions between PAMAM dendrimers in concentrated solutions using SANS and SAXS techniques. They showed that the molecules develop large scale interactions which can be screened by the addition of excess acid or electrolyte. SANS studies of the structural properties of poly(propyleneimine) dendrimers (DAB-*dendr*-$(NH_2)_{64}$) in D_2O examined the influence of the degree of ionization and solvent pH.[59,60] Upon addition of HCl, the dendrimers become charged and the scattering patterns exhibited a single correlation peak indicating a spatial arrangement of the molecules due to the electrostatic repulsion. In the uncharged state, these dendrimers behave as "soft" molecules, i.e., they have a very high degree of flexibility with possible interpenetration at high concentration. In conditions where they are charged, however, the electrostatic forces cause a stretching of the branches and reduce their flexibility, i.e., they behave as "hard" particles.

Changes in the G6 PAMAM structure upon pH titration were studied using a polarity-responsive probe by Chen, Tomalia, and Thomas.[61] Two possible models were proposed to explain the dendrimer behavior; in the first, some inward folding of the dendritic termini is presumed to occur at all basic pHs, resulting in a slightly less polar interior. At high pH (ca. pH 10) any protonation will occur on the more polar surface amines; as the pH is lowered to pH 8.3, the less polar amines will protonate and the average environmental polarity decreases, as was observed from the experimental measurements. Finally, further decrease of pH causes protonation of the internal amines and molecular expansion due to charge repulsion. In the alternative model, increasing protonation of dendritic termini is able to overcome entropy, forcing charged termini to the periphery; the resulting surface crowding results in the observed lower average environmental polarity. In this model, the polarity increase on decreasing pH below 8.3 arises from protonation of the amines that are located in the less dense, more polar dendritic interior. The first model is considered a denser core model of the G6 dendrimer, while the second is a denser shell model.

A detailed study of the physicochemical properties of G5 PAMAM dendrimers in aqueous solution by Nourse, Millar, and Minton[35] revealed contrasting properties of amino-surface (G5-NH_2) and hydroxyl-surface (G5-OH) dendrimers. The hydrodynamic properties of G5-OH in dilute solution could be described by a model in which the solute was represented by a single species of quasi-spherical particle having a molar mass equal to the theoretically predicted molar mass of the dendrimer and a hydrodynamic radius of about 3.1 nm. In contrast, size exclusion chromatography and sedimentation equilibrium ultracentrifugation studies on G5-NH_2 have indicated the formation of oligomeric aggregates in aqueous solution even in the presence of high salt concentration. Measurement of the concentration dependence of

sedimentation of G5-OH in pH 7.2 phosphate buffer indicated the presence of significant electrostatic repulsion overlaid on weakly attractive interactions leading to the formation of nonspecific aggregates at sufficiently high dendrimer concentration.

Static and dynamic light scattering studies by Milhem[58] have shown that G4 PAMAM dendrimers do not exhibit intermolecular aggregation in aqueous solution at concentrations up to 7% w/w at pH 10.5, or even when fully unionized at pH 14.

Internal Structure

Many of the potential applications of dendrimers depend directly on the organization and distribution of internal segment densities and the possibility of reduced density at the core. The first theoretical treatment of internal structure of the amine-terminated PAMAM dendrimers by de Gennes and Hervet[8] predicted a segment distribution function that has the highest density on the periphery and a relatively hollow core. On the other hand, Monte Carlo simulations by Lescanec and Muthukumar[62] predict that the highest density is at the center with a decaying profile to the edge of the molecule. Both the models have shortcomings; the de Gennes model does not account for backfolding, which could be an incorrect assumption for flexible dendrimers, whereas the Lescanec and Muthukumar model is based on kinetically grown rather than equilibrium structures. The predictions of these models have been tested against the experimental data from a variety of techniques.

The SAXS studies on methanolic solutions of G3 to 10 PAMAM dendrimers by Prosa et al.[52] indicate that lower generation dendrimers are less dense than their higher generation relatives, but after the first few generations the average density appears to be roughly independent of generation and uniform throughout the structure with no indication of any sizeable minimum in density near the dendrimer core (with the G10 dendrimer) contrary to the predictions of the de Gennes model.

Measurements of molecular density[39] and intrinsic viscosity[37,39] of PAMAM dendrimers indicate an unusual variation with dendrimer generation. Minimum density and maximum intrinsic viscosity were observed at around G4, which suggests that the fully developed dendrimers have a high accessible internal surface area in a solvent-filled intramolecular free volume that may consist of internal cavities and channels. Similar findings were reported by Mourey et al.[63] for PBE monodendrons (based on dihydroxybenzyl alcohol) and tridendrons produced by coupling these dendrons to a trifunctional core, 1,1,1-tris(4′-hydroxyphenyl)ethane prepared by the convergent method. A maximum intrinsic viscosity occurred at G3 for tridendrons and G5 for monodendrons, consistent with the model developed by Lescanec and Muthukumar that predicts inward folding of branch units, a maximum density in the center, and a distribution of terminal groups throughout the structure. If there are no specific interactions between the groups and if the branch units of the dendrimer are flexible enough to allow some gradual backfolding, then it is reasonable that the equilibrium structure has maximum density in the center because this corresponds to maximum entropy for the molecule and also provides relief of steric crowding of terminal groups. It is also significant that this model describes the behavior of both PAMAM and polyether dendrimers implying that it may be of general application to all flexible dendrimers.

A study of the molecular characteristics in dilute solution (D_2O, 1%) of the first five generations of the poly(propyleneimine) dendrimers DAB-*dendr*-$(NH_2)_x$ and DAB-*dendr*-$(CN)_x$, ($x = 4$, 8, 16, 32, and 64) by Scherrenberg et al.[64] has suggested some degree of backfolding of molecules within the dendrimer. Comparison with molecular dynamics calculations indicated that the poly(propyleneimine) dendrimers could be regarded as flexible molecules with a relatively homogeneous density distribution, not in line with either the de Gennes or Lescanec and Muthukumar models.

There is considerable evidence from a variety of techniques for a dendrimer model composed of a relatively soft or spongy interior surrounded by a considerably harder outer molecular surface, the so-called "dendritic box."[17] ^{13}C-NMR measurements of spin lattice relaxation times of specifically tagged PAMAM dendrimers[65–68] have shown considerably reduced mobility in the outer surface groups relative to the interior segments. Meltzer et al.[65,66] performed ^{13}C-NMR relaxation studies on hydroxyl terminated PAMAM dendrimers, in order to understand the influence of the end groups on the chain dynamics of the molecule (G0.5 to G10.5). The spin relaxation times (T_1) for each terminal carbon decreased continuously with an increase in generation, suggesting that segmental motion was increasingly restricted at the surface of the higher generations. Thus, it was concluded that the iterative branching process that creates the PAMAM architecture must at some point lead to severe steric crowding either at the molecular surface or throughout the volume of the molecule, as indeed is indicated from modeling of dendrimer growth. It was, however, noted that the mobility of the terminal groups on the surface of dendrimer molecules was higher than expected for the available area. Therefore, either the number of terminal groups must be much less than expected, possibly due to growth failure, or the conformation must be such that all the terminal groups do not reside at the surface due to their folding back into the interior of the molecule. Gorman et al.[69] also showed

that some terminal groups (approximately 3) of a G3 dendrimer were located very close to the dendrimer core. An average of 25 of the 32 terminal groups were found on the geometric periphery which were more mobile than the others found elsewhere in the dendrimer molecule.

Differential scanning calorimetric (DSC) measurements on a variety of dendrimers including PAMAM dendrimers with NH_3 and EDA cores,[39] and PBEs and phenolic-terminated polyesters[27] have shown exponentially increasing glass transition temperatures with increasing molecular weight reaching an asymptotic value generally at about G3 or G4. These results show that a pronounced segmental mobility is retained within the internal volume, possibly around the core and over the first two or three generations in support of the conclusions from ^{13}C-NMR relaxation studies. Moreover, at about the fifth branch layer around the core, segmental motions responsible for the glass transition apparently reach their limiting values and cannot be extended any further.

Flow Properties and Inter-dendrimer Interaction

The flow properties of solutions of dendrimers have relevance not only for their possible use in pharmaceutical formulation, but also because of the insight that might be gained on the nature of any intermolecular interactions between dendrimers. It might be expected from computer modeling of the dendrimer shape that lower generations, which have an open "plate-like" or "dome-like" entity, would readily permit a branch from a neighboring dendrimer to penetrate into the interior. This tendency would be further enhanced by intermolecular hydrogen bonds between the interior amide groups of two interpenetrating molecules or between the primary amine units of one molecule and the amide carbonyl oxygens of another. In contrast, the closure of the dendrimer outer surface at higher generations should result in minimal inter-dendrimer interactions.

Measurements by Uppuluri et al.[36] using a cone and plate rotational rheometer on the first seven generations of EDA-core PAMAM dendrimers in medium and highly concentrated solution (30%–75% in EDA) showed typical Newtonian flow behavior over the entire range of shear stress and strain examined. An absence of any abrupt change in the slope of log zero-shear viscosity against log weight average molecular weight relationship was indicative of a lack of any significant interpenetration of dendrimers to form quasi-networks even at such high concentration where any such inter-dendrimer interaction would be accentuated. Similar measurements on neat linear polyamidoamine illustrated the unique bulk flow properties of the dendrimers compared with a chain polymer of comparable molecular weight, which showed an inflection in these plots associated with the onset of chain entanglement. There is also a fundamental difference in the flow properties of these dendrimers and colloidal suspensions of spheroidal particles, which typically show shear thinning properties above some critical shear rate, molecular weight, or concentration originating from a tendency of individual particles to aggregate. The viscosities of the dendrimer solutions were at least an order of magnitude lower than those usually found for similar solutions of chain-type macromolecules of comparable molecular weights and concentrations, i.e., they flowed much more easily than their chain-type equivalents. It was proposed that the atypical flow properties of the dendrimers compared to other high molecular weight synthetic polymers were a consequence of the unique dendrimer architecture, which above the critical generation results in globular spheroids whose outer surfaces close upon themselves or at least become dense enough to be impenetrable for other dendrimers or large molecules, giving rise to the Newtonian flow properties of these solutions. The situation is thus the reverse of that of typical long-chain polymers, where the probability of entanglement increases with increase of chain length. A pronounced sensitivity of solution viscosity to temperature and a stability of the dendrimer solutions to repeated loading, observed by these workers, suggested surprising flexibility of the dendrimer interior, in agreement with their reported ability to expand[70] or shrink[71] depending on solvent quality, solution concentrations, and temperature. These observations support a model of the dendrimer having a soft or spongy interior and a surrounding denser more congested shell.

A detailed rheological examination of the first eight generations of PAMAM dendrimers[72] explored rheological behavior in the bulk (rather than solution) state under conditions of steady shear, shear creep, and dynamic oscillatory shear over a wide temperature range. A distinct change from single relaxation mode to a multirelaxation mode, Maxwell-type behavior at G4 was consistent with the soft interior-dense shell model involving the closure of the dendrimer molecular surface upon itself. Furthermore, an analysis of the free volume of PAMAM dendrimers revealed a dependence on generation that was consistent with the qualitative conformational change at G4 predicted by this model.

Further insight into the nature of the dendrimer surface has been provided by measurements of the melt viscosity of PBE monodendrons and tridendrons.[73] Logarithmic plots of melt viscosity against molecular weight were linear with a slope close to the expected value of unity and no detectable inflection attributable to onset of chain entanglement. These observations confirm that the melt viscosity behavior is not dominated by chain entanglements and is fully consistent with a globular highly

branched structure with an increasingly congested surface lacking intermolecular chain entanglements.

PHARMACEUTICAL APPLICATIONS OF DENDRIMERS

Encapsulation/Solubilization of Drugs by Dendrimers

The concept of encapsulating guest molecules into a special, egg shell-like structure was proposed by Maciejewski in 1982.[74] There has been a considerable interest in the use of dendrimers to encapsulate or interact with labile or poorly soluble drugs in order to protect the drug and enhance bioavailability. Such guest–host dendrimer systems have also been proposed as controlled delivery systems. Although several terms have been applied to systems in which drugs have been encapsulated within a dendritic structure, these systems are usually only distinguishable by subtle differences in dendrimer architecture. The interaction within the dendrimer may be simple physical entrapment, or can be more specific involving hydrophobic interactions or hydrogen bonding.

Dendritic structures having a hydrophobic core and hydrophilic surface layer have been described as unimolecular micelles.[14,15,75–79] However, unlike conventional micelles, the dendritic structure is independent of dendrimer concentration; thus, they do not possess a critical micelle concentration. Newkome et al. described a monomolecular micelle cascade polymer based on a neopentyl core and 36 carboxylic acid surface groups.[77] A number of lipophilic probes (e.g., phenol blue) were found to be associated within the lipophilic infrastructure of the micellanoates whose size ranged 3 nm–5 nm in diameter. Hawker et al. described the convergent synthesis of dendritic polyether unimolecular micelles based on an 3,5-dihydroxybenzyl alcohol building block with carboxylate surface groups.[14] The dendritic micelles were capable of specific nonbonding interactions ($\pi-\pi$ interactions in this case) through tailor-made molecular inclusion sites. The electron-rich macromolecular structures (Fig. 5) were able to solubilize a variety of polycyclic aromatic compounds in water. The solubilizing power of

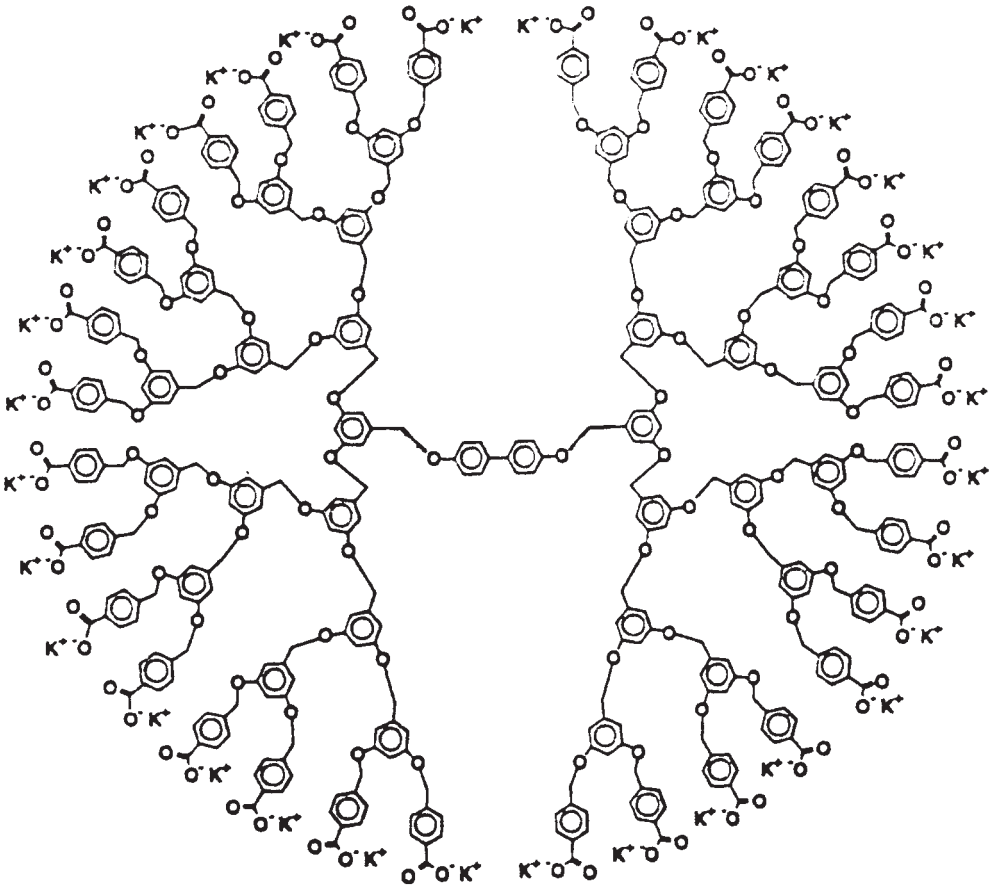

Fig. 5 Water soluble unimolecular dendritic polyether micelle. (From Ref. [14], © 1993 Royal Society of Chemistry.)

the dendrimer was found to increase with dendrimer concentration and also as the electron density of the polycyclic aromatic compounds increased. Hybrid dendritic structures were also described in which hydrophobic and hydrophilic chain ends were segregated at distinct ends of the globular structure, thus allowing for preferential orientation at certain interfaces. Dendrimers based on 3,5-dihydroxybenzyl alcohol have limited solubilization capacity as a result of small internal cavities within the dendrimer. Liu et al. synthesized water-soluble dendritic unimolecular micelles based on 4,4-bis(4′-hydroxyphenyl)pentanol building blocks and a surface shell of polyethylene glycol (PEG) chains.[15,32] The building block was chosen in order to increase the flexibility and cavity size of the dendritic structure while the PEG was chosen because of its good water solubility and demonstrated biocompatibility (Fig. 6). The solubilization capacity of the unimolecular micelle was demonstrated by the solubilization of pyrene (~ 365-fold increase in solubility) in aqueous solution and entrapment of a model drug (indomethacin) at a loading of 11% w/w (G3 micelle), a value that corresponds to approximately nine drug molecules per micelle. The drug-loaded dendrimer provided sustained release of indomethacin over a period of 30 hr.

Jansen et al. describe the entrapment of molecules in a dendritic box,[17,80–82] based on poly(propyleneimine) dendrimers with a chiral shell of protected amino acids (Fig. 7). The resulting dendritic structure, 5 nm in size, possesses a dense shell (as a result of bulky surface groups) with solid-phase character. Guest molecules were entrapped within the internal cavities of the dendrimer. The dense outer shell prevents diffusion from the dendritic box, even after solvent extraction, prolonged heating, or sonication. The entrapment effect was only seen with higher generation structures, e.g., a dendrimer with 64 amine end groups entrapped up to four Bengal Rose molecules. An extension of this work involved the shape-selective liberation of guests from dendritic boxes.[81] Hydrolysis of the surface t-BOC groups (with formic acid) of a dendritic box containing entrapped Bengal Rose and 4-nitrobenzoic acid resulted in perforation of the dendrimer

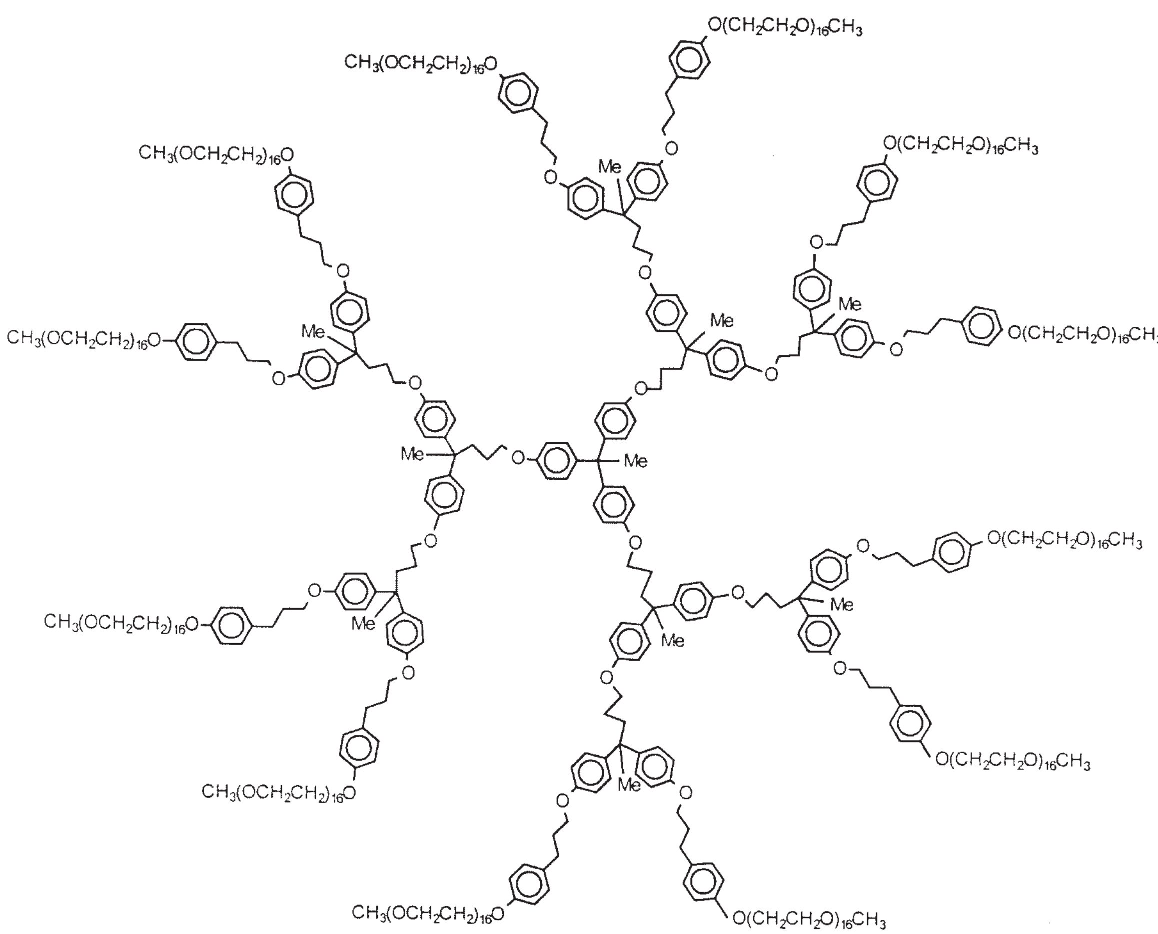

Fig. 6 G2 dendritic unimolecular micelle. (From Ref. [15], © 2000 Elsevier Science.)

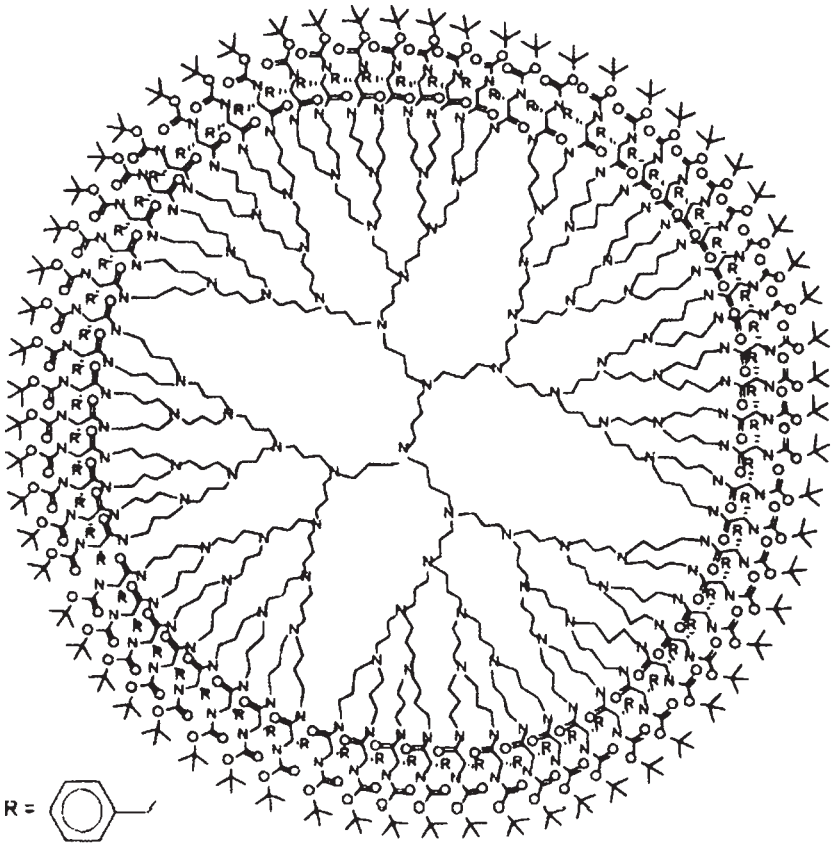

Fig. 7 Dendritic box based on poly(propyleneimine) dendrimer. (From Ref. [81], © 1995 American Chemical Society.)

allowing the release of 4-nitrobenzoic acid but not of Bengal Rose. The larger Bengal Rose molecule could only be liberated following hydrolysis of the outer shell by 12 M HCl under reflux for 2 hr.

An approach to increase the loading of guest molecules within a dendritic structure involves the creation of additional void space by removal of the dendrimer core, producing the so-called cored dendrimers.[12,83] This approach obviously requires a means of maintaining the structural integrity of a cored dendrimer and thus the connections among the dendritic wedges. The approach taken by Zimmerman's group was to extensively cross-link the dendrimer surface groups by a ring-closing metathesis reaction prior to removal of the core (Fig. 8). Core removal was via three cleavable ester bonds, with the remaining structure being unaffected as a consequence of robust ether linkages. Cored dendrimers have been compared to hollow polymeric nanospheres with the potential to encapsulate substances. It is not clear, however, how guest molecules could be loaded into such structures.

A recent report focusing on the encapsulation of drugs referred to two types of possible systems, such as the Dendrilock® and Dendripore® type structures.[84] The Dendrilock structure is based on a dendrimer with a congested outer shell, the release of guest molecules from which was either immeasurably slow or nonexistent. In contrast, Dendripores are lower generation dendritic host structures with less compact surfaces allowing time dependent release of guest molecules. The proposed mechanism of release is similar to that reported by Meijer's group,[17,80–82] i.e., dendrimer surface density is used as a means of controlling the release of guest molecules.

Kojima et al. synthesized G3 and G4 PAMAM dendrimers with PEG grafts and examined their ability to encapsulate the hydrophobic drugs adriamycin and methotrexate.[85] The modified PAMAM dendrimers were reported to have PEG grafted to every surface group and were estimated to have a hydrodynamic diameter of up to 14.7 nm. The purpose of the grafts was to improve biocompatibility and modify biodistribution. It was found that drug loading increased with dendrimer size and increasing chain length of PEG grafts, and up to 6.5 adriamycin or 26 methotrexate molecules could be

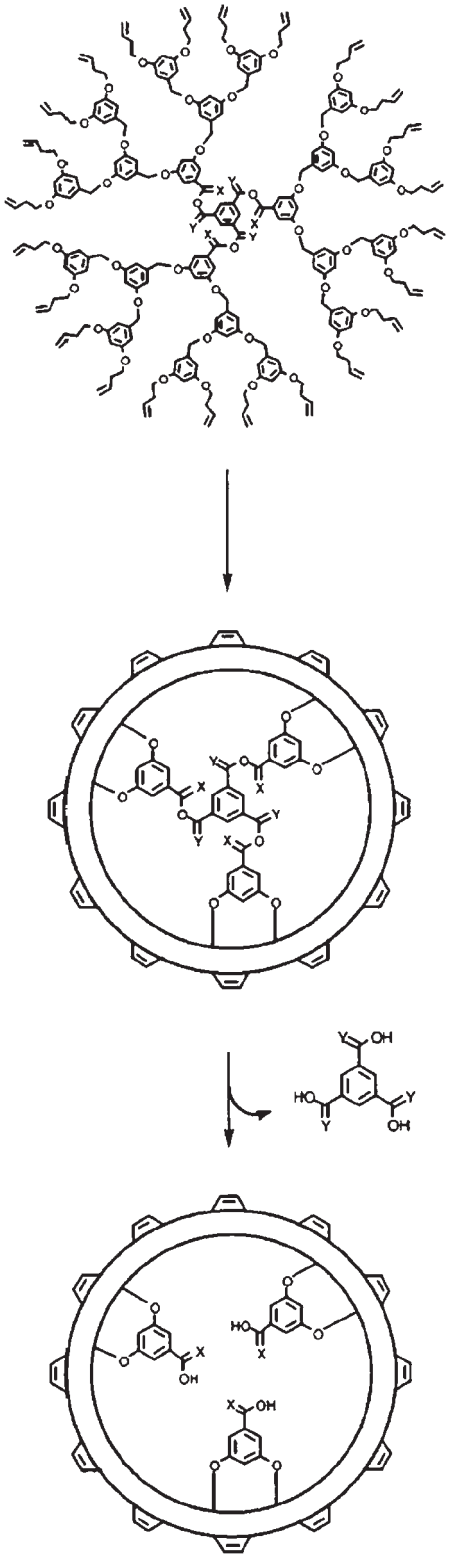

Fig. 8 Synthesis of a cored dendrimer. (From Ref. [12], © 1999 American Chemical Society.)

incorporated into a dendrimer. While there was evidence of the sustained release of methotrexate from a dendrimer carrier in an aqueous solution of low ionic strength, no control could be achieved in isotonic solutions.

Although the number of guest molecules incorporated into a dendrimer may be dependent to a limited extent on the architecture of a dendrimer, the loading capacity may be dramatically increased by the formation of a complex with the large number of groups on the dendrimer surface. The interaction between charged dendrimers and oppositely charged polyelectrolytes may result in the formation of soluble complexes.[86–91] Studies on the interaction of poorly soluble drugs have shown that solubility may be dramatically enhanced in the presence of dendrimer.[92–95] The solubility of ibuprofen, for example, was increased by a factor of over 140-fold in the presence of a 2% w/v PAMAM G4 dendrimer solution, over twice that achieved using a micellar solution of 2% w/v sodium dodecyl sulfate. The dendrimer–drug complexes were thought to involve an electrostatic interaction between the carboxyl group of the ibuprofen molecule and the amine groups on the dendrimer surface, though recent studies indicate the additional involvement of internal amine groups.[58] Although enhancing solubility, complexes formed as a result of electrostatic interactions may not provide sufficient control over drug release.[93] In order to provide a more controlled release of drugs, covalent conjugates have been synthesized between drugs and the numerous surface groups on a dendrimer. The hydrophobic drug ibuprofen was coupled onto the surface of G4 PAMAM dendrimers.[58,96,97] It was found that insoluble complexes were formed when more than five ibuprofen molecules were attached to the dendrimer surface. However, much higher drug loadings (up to 32 ibuprofen molecules per dendrimer) could be achieved when PEG chains were attached to the dendrimer. The release of ibuprofen from these dendrimer conjugates was examined. Liu et al. characterized a number of conjugates based on polyether dendrimers and a number of model drugs (cholesterol and two amino acid derivatives).[32] The solubility of the conjugates was maintained by grafting short PEG chains onto the dendrimer surface. Zhuo et al. synthesized a series of dendrimers with a branch structure similar to PAMAM dendrimers, but with a cyclic core of 1,4,7,10-tetraazacyclododecane.[34] The resultant dendrimer had four branches emanating from the core. 5-fluorouracil (5FU), conjugated onto the surface of the dendrimers, could be released by hydrolysis of the resulting conjugate.

Uptake of Dendrimers

A polymeric drug carrier such as a dendrimer must traverse through various cell barriers, i.e., epithelial and endothelial cells, before reaching its target. Transepithelial

transport is an important factor when considering movement across the intestinal cell wall, while transendothelial transport is relevant to the movement of carriers across the vasculature to reach their target cells/receptors. Generally, transport across biological barriers is determined by factors pertaining to both the nature of the carrier (e.g., drug molecule, macromolecule, and particle) and the nature of the biological barrier. Important carrier attributes are molecular weight, charge, hydrophobicity, flexibility, and the geometry of the molecule. Biological barrier attributes include the barrier's location and function; the blood–brain barrier is, for example, less permeable to molecules than the intestinal epithelium.

Proposed mechanisms of uptake of structures of dendritic size from the gastrointestinal tract include persorption, endocytosis by enterocytes, paracellular transport, uptake by intestinal macrophages and uptake through the gut-associated lymphoid tissue.[98] Wiwattanapatapee et al.[99] investigated the potential of 3 nm–7 nm diameter anionic and cationic PAMAM dendrimers to transverse an everted rat intestinal system. It was found that the uptake of PAMAM dendrimers was most likely to occur across enterocytes by transcytosis. It was also reported that cationic (full generation) PAMAM dendrimer are retained in the tissue. This retention could be a consequence of adsorption of positively charged dendrimers (as well as other polycations) onto negatively charged cell membranes, an effect also reported by Katchalsky.[100] Anionic PAMAMs were found to have high serosal transfer rates with potential as an oral drug delivery system.

Florence and coworkers studied the uptake, after oral administration, of a G4 poly(lysine) dendrimer (diameter = 5 nm) modified with a lipid surface.[98,101] In vivo studies indicated that there was a rapid uptake of dendrimers from the gastrointestinal tract, demonstrating their potential as particulate delivery systems. It was found that uptake of the dendrimers was not uniform along the gastrointestinal tract; there was preferential uptake through lymphoid tissue in the small intestine, but no uptake in lymphoid tissue in the large intestine. Peyer's patches in the small intestine preferentially absorb dendrimers over enterocytes, whereas the opposite is true in the large intestine. The level of uptake of the dendrimers was lower than that exhibited by polystyrene particles in the size range 50 nm–3000 nm. It was suggested that there was an optimum size for nanoparticulate uptake by the gut and that a reduction in size did not necessarily correlate with an increase in uptake.

Tajarobi et al.[102] studied the transport of a series of full generation PAMAM dendrimers (G0 to G4) across Madin-Darby canine kidney (MDCK) cells. It was found that the permeability of dendrimer across these cells was in the order G4 $\gg$ G1 $>$ G0 $>$ G3 $>$ G2 and was governed by the balance between the size of the dendrimer and its interaction with the cells. One of the factors attributing to the high permeability of the G4 dendrimer was the fact that it compromised cell integrity. Zhang and Smith[103] also showed that higher generation PAMAM dendrimers may disrupt or influence the integrity of the cells.

The factors influencing the extravasation of PAMAM dendrimers across microvascular network endothelium was reported by El-Sayed et al.[104] It was concluded that the rate of transport is dependent on size and molecular weight, as well as physicochemical properties such as geometry and charge. An increase in size of PAMAM dendrimers resulted in a corresponding exponential increase in extravasation time. Cationic dendrimers interact with the negative cell walls, and their extravasation time is therefore increased. The balance between charge and geometry was illustrated by the fact that at physiological pH, the polycationic PAMAM dendrimers exhibit faster extravasation than neutral PEG molecules of the same radius.

A certain degree of caution is required in the interpretation of uptake data where dendrimers are conjugated to a label. As reported by Yoo and Juliano, a dendrimer labeled with a fluorescent dye (Oregon Green 488) was a much better delivery agent for antisense compounds than unmodified dendrimer.[105] It is also important to fully characterize dendrimers as there is a concern regarding polydispersity, particularly with higher generation dendrimers.[103,104]

Delivery of DNA and Oligonucleotides by Dendrimers

Dendrimers have emerged as efficient carriers of DNA for the transfection of cells.[29,106–115] A study by Haensler and Szoka[106] demonstrated the high efficiency transfection of a variety of suspension and adherent cultured mammalian cells using plasmid–PAMAM dendrimer complexes. A maximal value of transfection was obtained by varying complex size and plasmid/dendrimer ratio. Expression was found to be unaffected by lysomotrophic agents and transfection efficiency was increased by over two orders of magnitude when a membrane-destabilizing peptide was attached to the dendrimer. The high transfection efficiency of dendrimers was believed to be related to dendrimer size, shape, and ability to buffer pH change in the endosomal compartment. The dendrimers were found to be well tolerated by cells and less toxic than poly(lysine). It is believed that polycations such as dendrimers act as a scaffold to condense DNA (the G6 dendrimer used in the study has a diameter similar to the histone core of chromatin, 7 nm). Efficient internalization is a result of the net positive charge of the DNA–polycation complex interacting with negatively charged cell-surface groups. Kukowska-Latallo et al.[107] reported much higher levels of

transfection mediated by dendrimer compared to poly(-lysine), and noted that different types of cell might be specifically transfected with different types of dendrimers depending on the cellular metabolism of the DNA–dendrimer complex. Lysomotrophic agents were found to have a beneficial effect on transfection. The difference in levels of transfection achieved in the two studies was attributed to the possible presence of impurities in the commercial dendrimer preparations used in the earlier study by Haensler and Szoka. A subsequent study from the Szoka group[29] reported that monodisperse PAMAM dendrimers in fact produce low levels of transfection whereas partially degraded dendrimers produced much higher levels of transfection ($>$ 50-fold). The higher levels of transfection activity were attributed to the high molecular weight degraded component (fractured dendrimer) being less sterically constrained. The increased flexibility of the dendrimer branches enables the fractured dendrimer to be compact when complexed with DNA and to swell when released from DNA in cells. A comparison of the transfection efficiency of dendrimers to a range of polycationic gene transfer agents indicated that degraded dendrimers displayed high transfection activity with relatively low cytotoxicity.[116] Polyethylene glycol conjugated low generation PAMAM dendrimers have been shown to be more efficient transfection agents than partially degraded (fractured) dendrimers.[115] It is believed that the presence of PEG chains on PAMAM dendrimers would mimic some of the essential properties of fractured high generation dendrimers (enhanced flexibility of chains). The use of lower generation dendrimers and the addition of PEG chains also enhances biocompatibility.

Dendrimers have been successfully used as a delivery system for antisense oligonucleotides (ODNs).[105,117–120] Hughes et al.[117] reported the significant enhancement of the effectiveness of antisense ODNs in the presence of nontoxic concentrations of G5 PAMAM dendrimer. Bielinska et al.[118] reported the enhanced activity of antisense ODNs and antisense cDNA plasmids in the presence of PAMAM dendrimers in an in vitro cell culture system. Dendrimer was believed to enhance the transfer of ODN into cells. The electrostatic binding of the phosphodiester ODNs to dendrimers also extended their intracellular survival. Dendrimers at concentrations required to be effective ODN carriers were reported to be noncytotoxic.

Cancer Therapy Based on a Dendritic Platform

A number of studies have examined the use of a dendrimer drug carrier to treat a variety of tumors. One approach has been based on the exploitation of the enhanced permeability and retention effect (EPR effect) to localize drug conjugates in tumor tissue.[121] A second approach has involved the conjugation of a drug-loaded dendrimer to a targeting moiety such as an antibody or a receptor-specific molecule (ligand) to target specific tumor cells. Structurally, dendrimers are multibranched and can therefore carry a relatively large payload of drug, as well as other bioactive macromolecules on their surfaces or interior. They are also monodisperse systems and the size of conjugates may be calculated with relative accuracy.

Cisplatin is an important anticancer agent, but as with other platinate drugs it suffers from low water solubility, severe toxic side effects, and the inherent or acquired resistance seen in a number of tumors. Malik et al. conjugated a G3.5 PAMAM dendrimer with a carboxylate surface to cisplatin to produce a dendrimer–platinate that had a high drug loading ($\sim$ 25%), was highly soluble and released platinum slowly in vitro.[122] In vivo studies demonstrated that intraperitoneal administered dendrimer–platinate and cisplatin were equally effective in mice bearing L1210 tumors, whereas only the dendrimer–platinate was active in mice bearing B16F10 tumors. When administered intravenously to treat a palpable subcutaneous melanoma, the dendrimer–platinate displayed antitumor activity, whereas the cisplatin was inactive. It was shown that the dendrimer–platinate selectively accumulated in solid tumor tissue by the EPR effect, and that the conjugate was also significantly less toxic than cisplatin.

Kono, Liu, and Fréchet[123] reported the synthesis of polyether-based dendrimers conjugated to methotrexate or folate residues, which may be used to target the folate receptor that is overexpressed in almost all solid tumors. Wiener et al.[124] also used folate-conjugated PAMAM dendrimers to target tumors, and showed that these dendrimers were accumulated in cells in a receptor specific manner.

Other examples exist of dendrimers (particularly PAMAM dendrimers) being conjugated to antibodies to target tumors. Barth et al.[125] synthesized boronated PAMAM dendrimers conjugated to the IB16-6 monoclonal antibody, which is directed against the murine B16 melanoma. The conjugate was designed for boron neutron capture therapy. The immunoconjugates showed a high level of immunoreactivity in vitro, but in vivo were localized in the liver and spleen. The same group conjugated epidermal growth factor (EGF) onto a boronated G4 PAMAM dendrimer to target brain tumors.[126] When administered intrathecally the conjugate was selectively delivered to EGF receptor positive gliomas, but when delivered intravenously the conjugates were once again localized in the liver and spleen, and very little crossed the blood–brain barrier. Abu-Rmaileh et al. proposed a drug loaded PAMAM based dendrimer conjugated to an antibody fragment specific to tumor cells bearing the carcinoembryonic antigen.[127]

The conjugates had PEG chains grafted onto their surface to minimize uptake by the reticuloendothelial system. Kobayashi et al.[128] demonstrated that monoclonal antibodies may be conjugated to dendrimers with minimal loss of immunoreactivity. There have also been a number of reports on the conjugation of dendrimers to antibodies for use in immunoassay.[129,130]

Baker et al. discussed the technical requirements of a smart nano-device therapeutic to diagnose and treat cancer and proposed two approaches.[131] The first involved the attachment of all the active moieties required for functionality to the same dendrimer, while the second involved the construction of a dendrimer cluster of many dendrimers each carrying a different functional group (Fig. 9). A G5-Folate-FITC conjugate was synthesized and used to successfully target cell lines expressing the folate receptor.

Biological Evaluation of Dendrimers

While several pharmaceutical applications for dendrimers have been proposed, there is limited evidence to suggest that they are safe for human use. This may be partly due to difficulties in assessing these materials. An ongoing concern in dendrimer science is sample purity, and it is not a simple procedure to unambiguously establish the purity of high-generation dendrimers.[103,104] It is likely that new dendrimers will be developed from building blocks known to be biocompatible or degradable in vivo to natural metabolites, e.g., the poly(glycerol-succinic acid) dendrimers as described by Carnahan and Grinstaff.[132] Information on dendrimer toxicity and biocompatibility has been included in a number of studies,[99,102,106,107,115,117,125,126] but there are only two reports that specifically address the safety of dendrimers. Roberts, Bhalgat, and Zera[133] investigated G3, G5, and G7 PAMAM dendrimers. The dendrimers were tested in V79 cells and Swiss-Webster mice for a number of biological properties including toxicity, immunogenicity, and biodistribution. Biological complications were observed only with G7 dendrimers at high concentrations. The biodistribution of the dendrimers was generation dependent; G3 showed the highest accumulation in kidney tissue, whereas G5 and G7 preferentially localized in the pancreas. Although it was concluded that the dendrimers did not exhibit properties that would preclude their use in biological applications, it was stressed that the biodistribution of dendrimer preparations should be carefully evaluated. The authors also noted that the dendrimers were not pure (yield ranged 62.7%–76.7%). Malik et al.[134,135] examined the biocompatibility of PAMAM, poly(propyleneimine) with either diaminobutane or diaminoethane as core, and poly(ethylene oxide) (PEO) grafted carbosilane (CSi-PEO) dendrimers. These dendrimers were used to systematically study the effect of dendrimer generation and surface functionality on biological properties in vitro. It was reported that dendrimers bearing amine surface groups showed concentration-dependent hemolysis, and changes in red cell morphology were observed. CSi-PEO dendrimers and those dendrimers with carboxylate terminal groups were neither hemolytic nor cytotoxic towards a panel of cell lines in vitro. In general, cationic dendrimers were found to be cytotoxic. Preliminary studies with polyether dendrimers showed that dendrimers with carboxylate and malonate surfaces were not hemolytic at 1 hr, but unlike anionic PAMAM dendrimers they were lytic after 24 hr. Cationic ^{125}I-labeled PAMAM

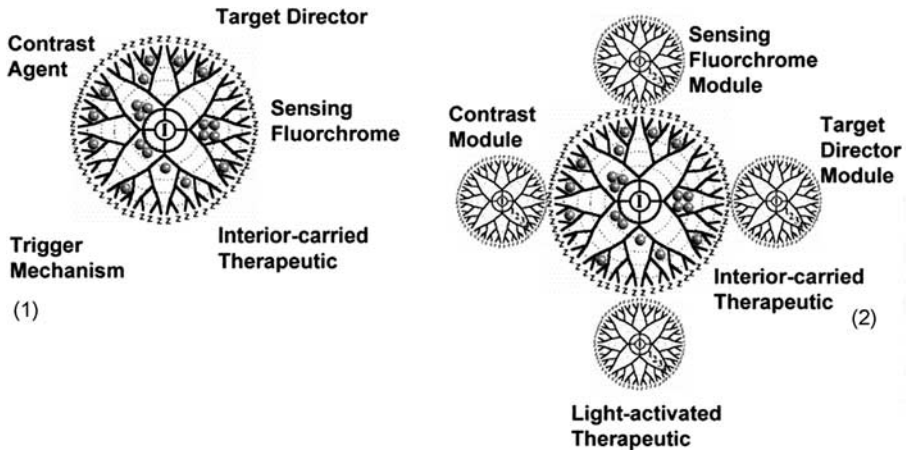

Fig. 9 Two alternative designs for a dendritic nanodevice cancer therapeutic. Configuration 1 is a therapeutic agent built around a single dendrimer molecule (70 Å in diameter). Configuration 2 is a cluster reagent where multiple dendrimers are clustered together, each providing a different functional unit (approx. 200 Å in diameter). The advantage to this latter approach is that functional units can be mixed and matched in a combinatory method to address almost any type of cancer. (From Ref. [131], © 2001 Kluwer Academic Publishers.)

dendrimers (G3 and G4) administered i.v. to Wistar rats were cleared rapidly from the circulation. Anionic PAMAM dendrimers (G2.5, 3.5, and 5.5) showed longer circulation times with generation-dependent clearance rates. In general, lower generation dendrimers had longer circulation times. The inherent toxicity of the higher generation cationic dendrimers suggest they are unlikely to be suitable for parenteral administration, especially if they are to be used at a high dose. The results indicated that dendrimer structure must also be carefully tailored to avoid rapid hepatic uptake if targeting elsewhere is a primary objective. The study did not consider the issue of dendrimer purity. It is not clear whether the concentrations reported are clinically relevant. In both of the above studies, unmodified dendrimers were evaluated. However, the biological profile of a dendrimer-based delivery system (with surface modifiers and a payload of drug) is likely to be different. It has been shown that DNA–PAMAM dendrimer complexes are less mytotoxic than free dendrimer, possibly due to the fact that the complex reduces the overall positive charge of the dendrimer.[136] The addition of PEG chains to PAMAM conjugates has been found to increase circulation times and modify biodistribution, with less conjugate being accumulated in the liver and kidney.[137]

CONCLUSIONS

Dendrimers are a unique class of hyperbranched polymers with well-defined size, shape, and chemical functionality and with properties not found in classical linear and cross-linked polymers. There have been significant developments in the last decade in many areas of dendrimer research, partly due to the commercial availability of dendrimers such as PAMAM, but also the result of the synthesis of several novel dendritic structures. Numerous pharmaceutical applications have been proposed for these hyperbranched polymers, and given the rate of current developments, it is envisaged that dendrimer-based formulations will appear in the next decade.

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